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TITLE: TP53 Synthetic Lethal Screen in Organoid Avatars to Discover Novel Therapeutic Targets for Colon Cancer

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14. ABSTRACT: Background: This application addresses the FY18 PRCRP topic area of Colorectal Cancer. Military relevant risk factors such as ionizing radiation often produce cancers with mutations in TP53. This proposal seeks to address a gap in colorectal cancer treatment that has profound impact on the health and well-being of military service Veterans, their beneficiaries, and extends to the American public as a whole. TP53 mutations are common in colon cancers (50%), and most often lead to resistance to chemotherapy. A systematic screen for synthetic lethal interactions between gene knockout, TP53 mutation, and chemotherapy such as Cisplatin will produce novel targets useful for treating a large fraction of colon cancers.								
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Introduction.

This progress report describes our work up to 02132023.

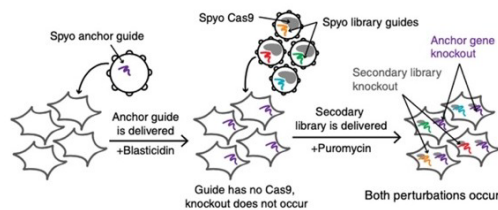
The overall progress was hindered by disruptions caused by the global SARS-CoV2 pandemic. This event caused a shutdown of the lab operations, loss of key personnel, and significant delays due to repeated COVID illness of the PI. Supply chain issues severely compromised progress during 2021-2022. The request for a second no cost extension has been granted and work is still continuing toward the original aims of the grant, however this final report is an administrative requirement and is being submitted (late) today in spite of ongoing work.

Keywords: Colon Cancer. TP53. Drug Resistance. CRISPR. Gene Knockout. Synthetic Lethal Interaction.

Accomplishments.

1. We used the strategy described by DeWeirdt, et al to screen a Brunello library in organoids. Our first successful screen was the control screen of TP53 Knockout Organoids in the absence of drug.

Genetic Screening for Studying Synthetic Lethality



2. Darwinian Fitness during 2 months of chemotherapy selection. Identify somatic mutation evolution under pressure.

12 organoids have been bar coded with individual isolated CLONTRACER library barcode clones and these bar codes are being used to track Darwinian fitness of mixtures of organoids treated with chemotherapies. These bar codes form a simple proxy for private somatic mutations located at different positions in the genome, and standardize the ability to track evolution in mixtures. This is a much

improved strategy for assessing evolutionary advantages among mixtures of organoids.

We are determining IC50s for select chemotherapies as a function of TP53 gene disruption.

The first set of compounds tested (Cisplatin, Oxaliplatin, Irinotecan, Olaparib) did not show the expected result of TP53 mutation causing resistance (as was observed in preliminary data). For this reason we are screening our TP53 WT and KO pairs of organoids against the entire set of drugs in the NCI Approved Oncology Drug Set IV, to identify additional candidate drugs to use during the screen for reversion. The Brunello library screen in the absence of drug has already yielded expected dropouts, and viable cultures of these library-infected organoids are frozen in liquid nitrogen and ready to conduct a sequential screen upon addition of chemotherapies identified in the above mentioned AODIV screen. We anticipate this last portion to be complete during the upcoming no cost extension (2023).

Impact.

Technical advances in library screening in organoids (clonagenic survival, large scale culturing, and bar coding for mixture tracking) have overcome barriers to library screening in organoids. Screen in the absence of drug is complete and the bar-code infected organoids (sans dropouts that happened without drug) is ready to culture in the presence of the correct chemotherapy agent.

Changes/Problems

The pandemic of 2020 caused by SARS-CoV-2 emerging from Wuhan China has had substantial negative impact on this project. Key personnel were lost during the shutdown and operations were harmed by supply chain compression created by the lockdowns. The PI has been infected with SC2 four times (hospitalized once with Delta) and this has caused significant delays.

Changes going forward.

An additional one year extension is requested.

Products.


NA

Participants and other collaborating organizations

NA

Special Reporting Requirements

NA

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